Fundamentals of Protein Chemistry
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Module - 09
Membrane Proteins and Transport
Lecture - 43
Membrane Transport - I

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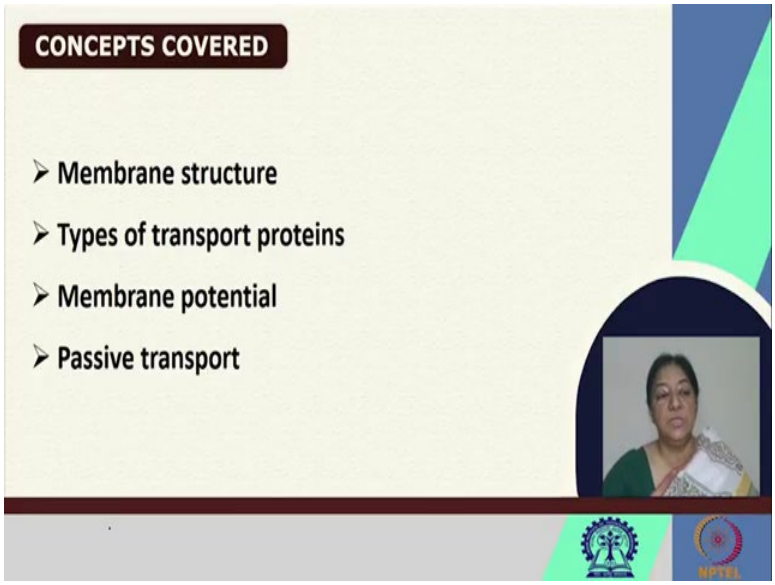

We begin our discussion on membrane transport. In the previous two lectures we looked at membrane proteins, how they are embedded in the membrane and the different types that are possible. In the next two lectures we will look at the types of transport proteins, what we mean by membrane potential and look at passive transport in this lecture and in the next lecture, we will look at active transport and discuss a few problems.

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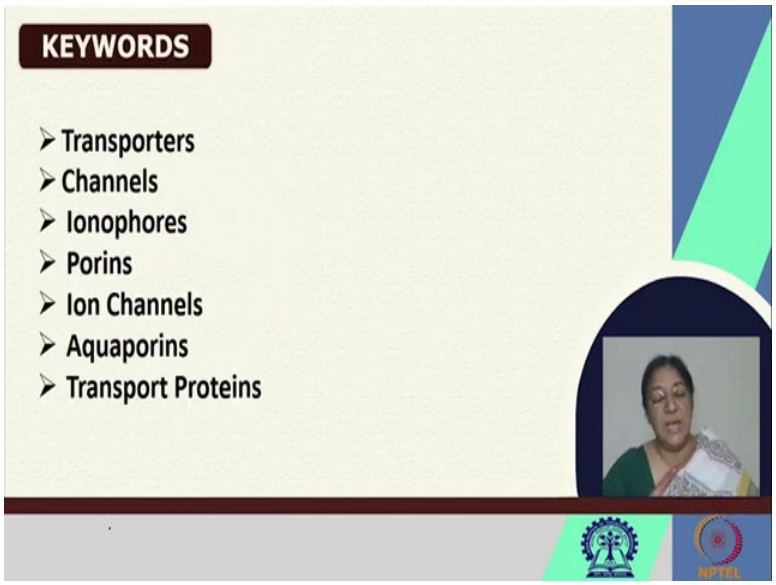

What we have as transporters, channels, ionophores, porins, ion channels are what mediate channel transport, membrane transport and are extremely important in the passage of nutrients and ions through the cell membrane.

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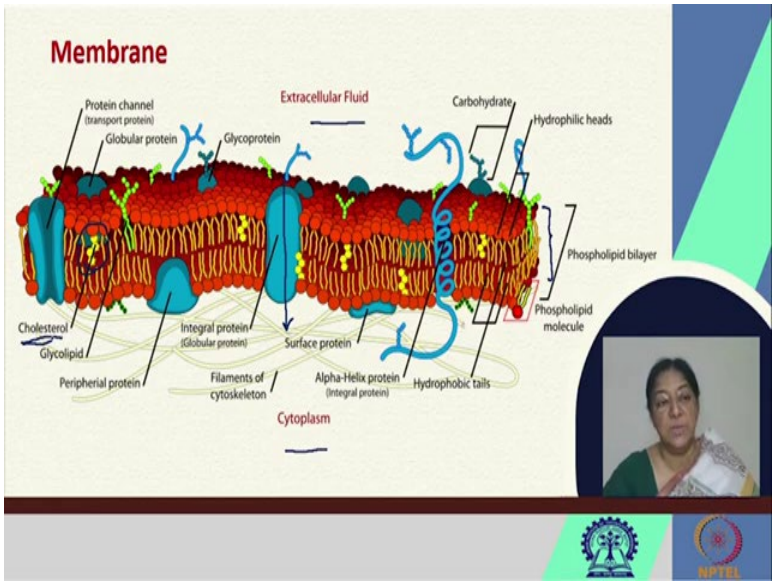

We looked at the composition of the phospholipid bilayer and found out they were the specific types of proteins that were required for the transport of material from the external extracellular fluid to the cytoplasm and these were integral proteins that could allow a transfer. These transfer proteins could be an α-helix type or a β-sheet type that formed a β barrel like in the coding proteins or we found a β-helix type that was there, to accommodate the specific transportation of ions.

The presence of cholesterol in these membranes gives a relative fluidity to the membrane, that is required for the motion and for a geometric conformational change that is going to be required for the ion transport or the transport across the membrane.

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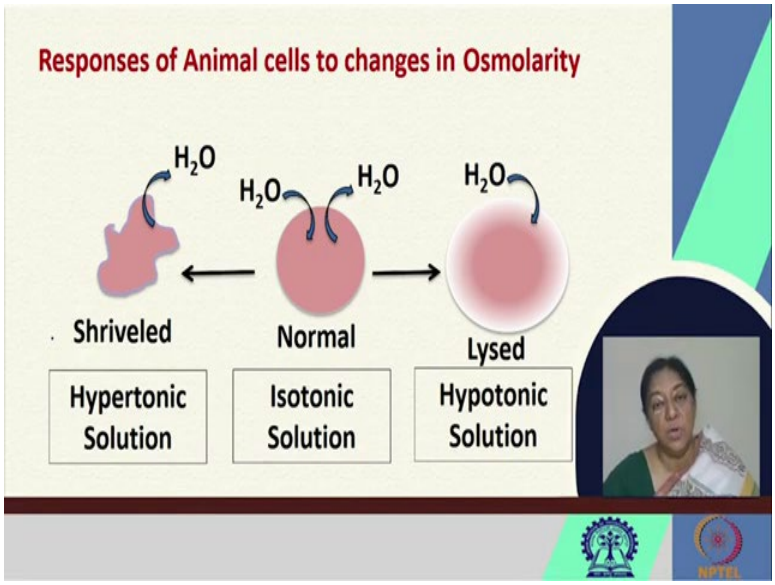

If we look at the responses of an animal cell to changes in osmolarity. If we have a normal isotonic solution, there is the transfer of water in and out of the system of the cell. However in a hypertonic situation, the water is going to be extracted out of the cell which will result in a shriveled cell.

In the opposite case where we have a hypotonic solution, then there will be water that would enter the cell and finally lead to its rupture. So, the condition that the transfer of water is perfected based on the membrane quality, is important here.

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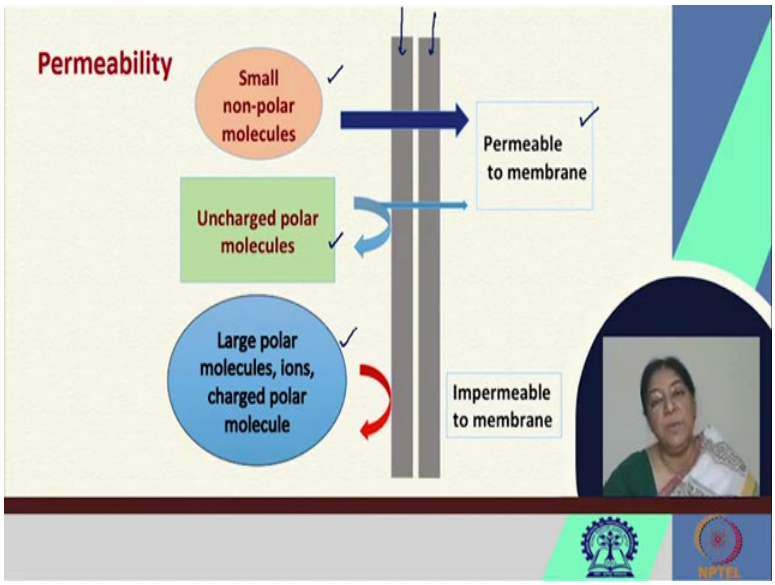

So, we look at the concept of membrane permeability. When we look at membrane permeability, we understand that this [refer to slide] is our lipid bilayer. We can have small polar molecules that would be permeable to the membrane. There could be uncharged polar molecules that may or may not pass through the membrane.

However, there could be very large polar molecules, other ions and charged polar molecules that would be impermeable to the membrane. Their transfer can be affected by specific transporter proteins and specific types of functions, that would bring about the process of the transfer from the extracellular material to the inside of the cell and from the inside of the cell to the outside.

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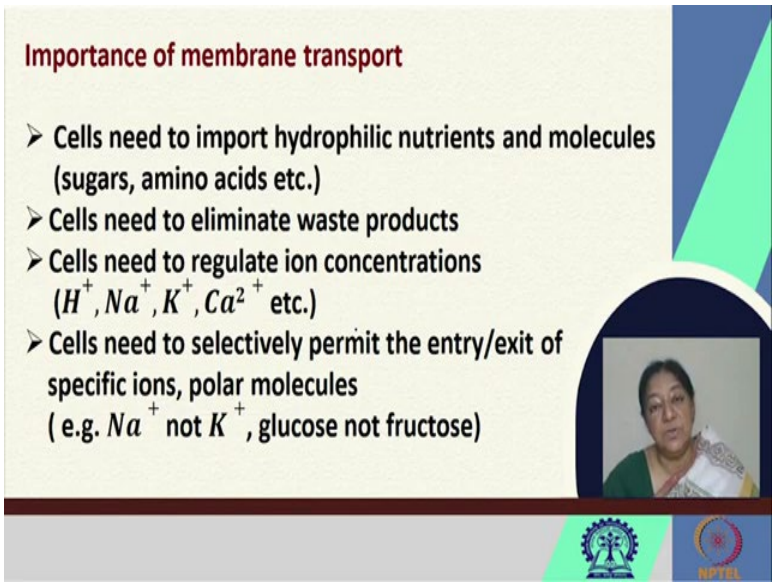

The importance of membrane transport therefore occurs because the cells need to import hydrophilic nutrients and molecules for the functioning, for the specific health of the cell to

maintain all the biochemical processes that go on into the cell. They also need to eliminate waste products, to regulate ion concentrations. These ions are needed for specific reactions that go on in the cell. Then they also need to selectively permit the entry and exit of specific ions or polar molecules.

For example, there may be a channel that allows the transport of sodium, but not potassium or glucose only and not fructose. So, this selectivity is very important for the maintenance of the cell.

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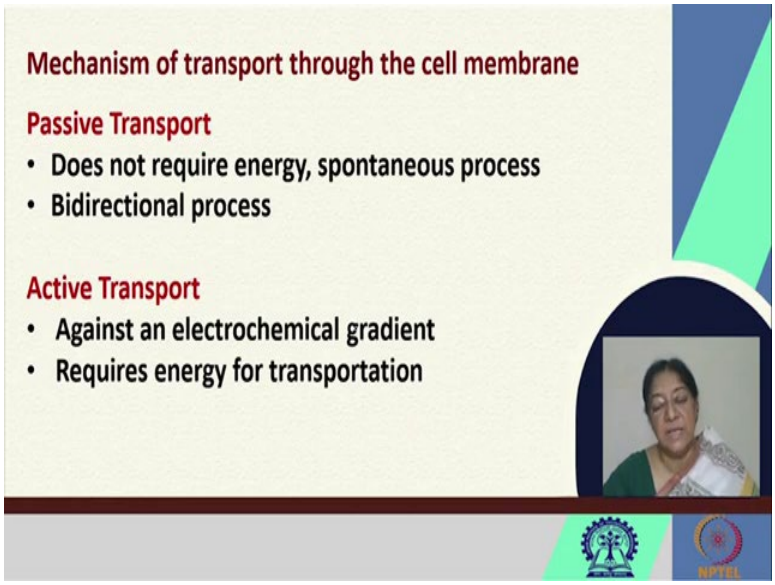

In the mechanism of transport through the cell membrane, it is possible that there is passive transport, where there is no extra energy required to bring about this transfer. It is a spontaneous process and commonly a bidirectional process. Active transport on the other hand, is against an electrochemical gradient and it requires energy for transportation. We will see what we mean by this chemical and electrochemical gradient, as we go on through the lectures.

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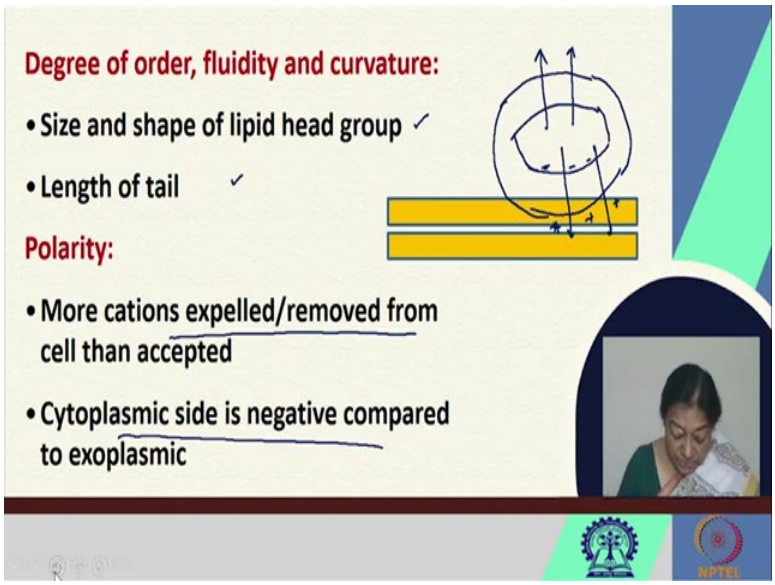

We looked at how the curvature can change in a lipid bilayer based on the size of the head group and the length of the fatty acid chains. So, the size and shape of the lipid head group and the length of the tail, was important to describe the fluidity of the membrane, which eventually led to the curvature, to the different leaflets that were part of the membrane. If we look at the polarity of the membrane, we see the curvature depends upon the type and size of the lipid head group and the length of the tail.

The polarity on the other hand, evolves because there are more cations that are expelled or removed from the cell, than accepted. So if there are more cations that are expelled, what happens is the cytoplasmic side is slightly negative compared to the exoplasmic side. So if the cations are selectively removed from the cell, then eventually what happens, there is a negative charge in this cytoplasmic region compared to the outside, which is slightly positive in nature.

This does not mean that it actually disrupts the electron neutrality in the sense, but this [refer to slide] this positive and this negative are relatively close together in the membrane, but because of the expulsion of the cations, this is slightly negative in nature.

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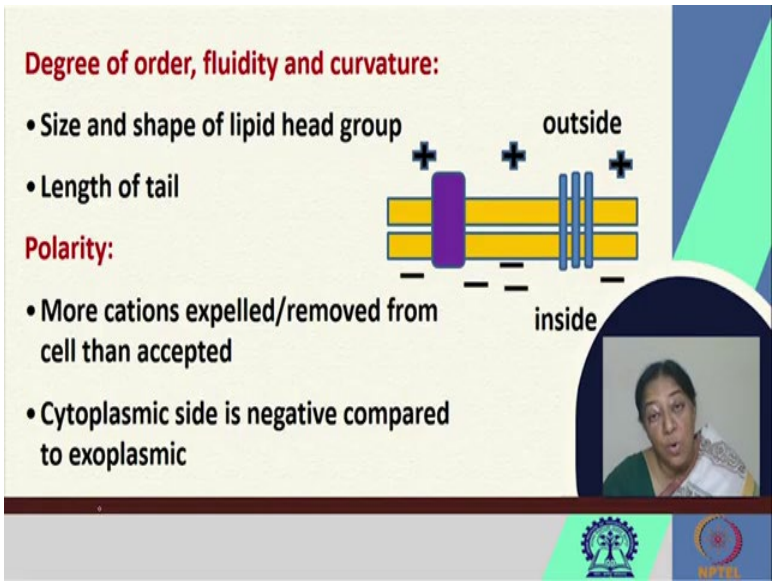

So we have our cell, we have a lipid bilayer, we have our membrane that could be say, a helical type or a porin type and on the outside we have positive charges, on the inside we have relatively more negative charges.

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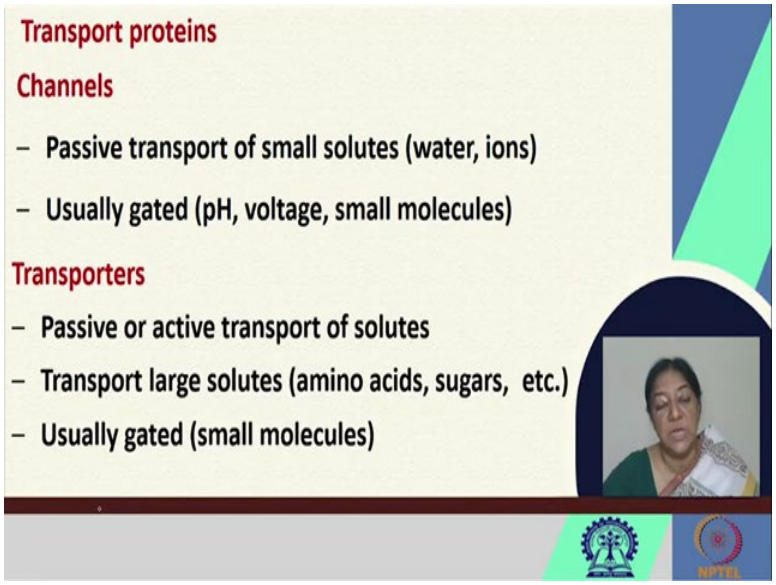

So in the transport proteins, there would be channels and there would be transporters that affect the transportation of nutrients in either direction of the cell membrane. When we are looking at passive transport of small molecules; water or ions, we can have gated channels.

These gated channels or these gates open when they are triggered by pH, by voltage or by small molecules. Similarly if we look at transporters, this allows the passive or active transport of solutes. Transportation of larger molecules are usually gated and there are small molecules that trigger the gates.

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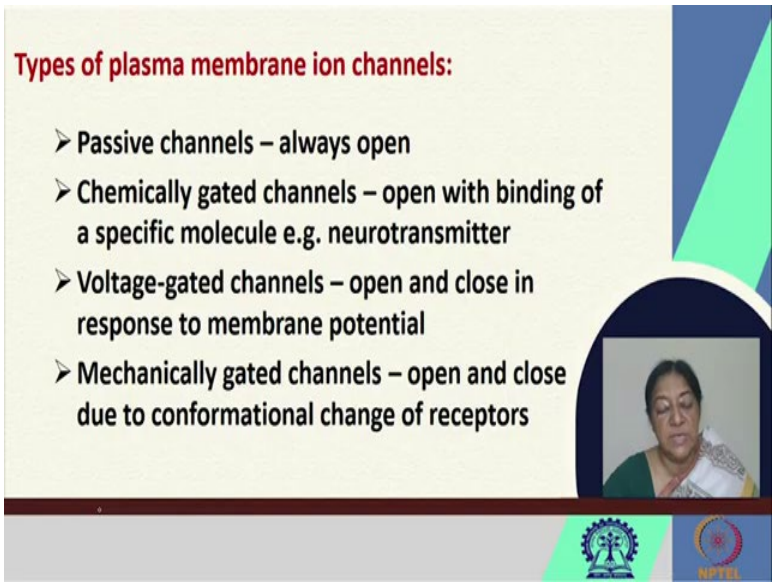

The types of plasma membrane ion channels that are possible, are passive channels. These are always open in the diffusion, possible for smaller molecules. There can be chemically gated channels, which open with say the binding of a specific molecule for example, a neurotransmitter.

They can be voltage gated channels that open and close in response to membrane potential. The changes in membrane potential may come across when there are ion transports; cation and anion transports, that could lead to a disruption in the membrane potential.

Then there are mechanically gated channels that open and close due to conformational change of receptors, that is possible again due to small molecule or large molecule binding, in or outside the specific protein that forms the transport protein.

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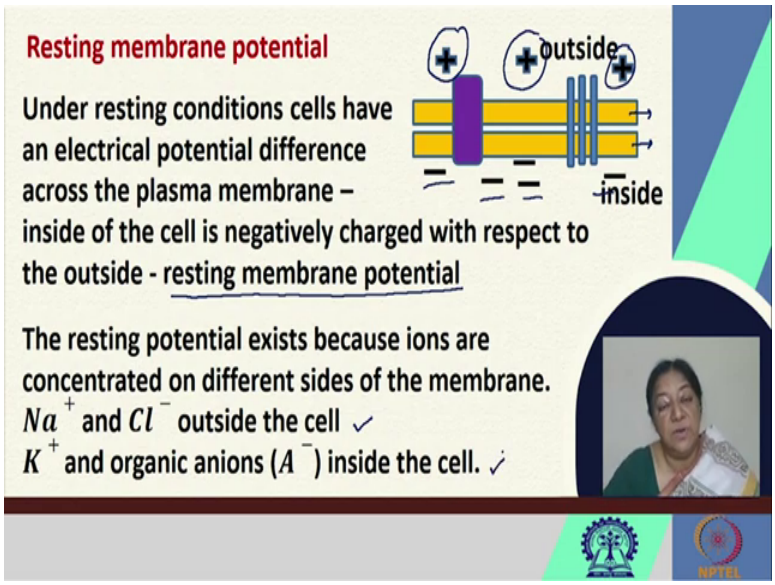

For the membrane potential, if we try and understand what we mean by a resting membrane potential, we saw the lipid bilayer. So, these [refer to slide] are the two leaflets that we have and on the outside level we saw there were more positive ions and inside, there were relatively more negative ions because of the expulsion of cations, preferably from the inside of the cell.

Under resting conditions therefore, cells would generate an electrical potential difference across the plasma membrane because the inside of the cell is negatively charged, with respect to the outside. This is called the resting membrane potential and this exists because of the disbalance of charges across the plasma membrane.

The resting potentia, therefore, exists because ions are concentrated on different sides of the membrane and for example, we could have Na^+ and Cl^- outside the cell and K^+ and organic ions generally written as A^-, inside the cell. This difference in the charges across the cell membrane, gives us what is called a resting membrane potential.

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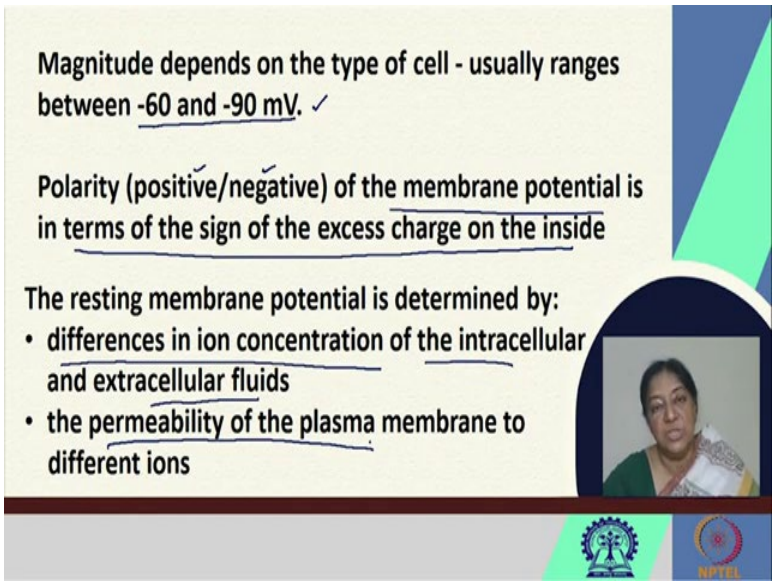

This magnitude depends upon the type of the cell and it usually ranges between -60 and -90 mV and the negative charge is because we have the excess charge on the inside of the polarity, the positive or the negative of the membrane potential, is in terms of the sign of the excess charge on the inside.

This is the way it is represented by convention, that the polarity, whether it is positive or negative, of the membrane potential is in terms of the sign of the excess charge on the inside and this as we saw is usually negative and the value ranges from around -60 mV to -90 mV.

Now the resting chemical membrane potential is therefore, determined by the differences in ion concentrations of the intracellular and extracellular fluids as can be understood and also, to the permeability of the plasma membrane to the different ions, which is important in bringing about this potential across the membranes.

So the resting membrane potential, would depend upon the differences that arise due to the differences in ion concentration in the intracellular and extracellular fluids and to the permeability of the plasma membrane to the specific ions.

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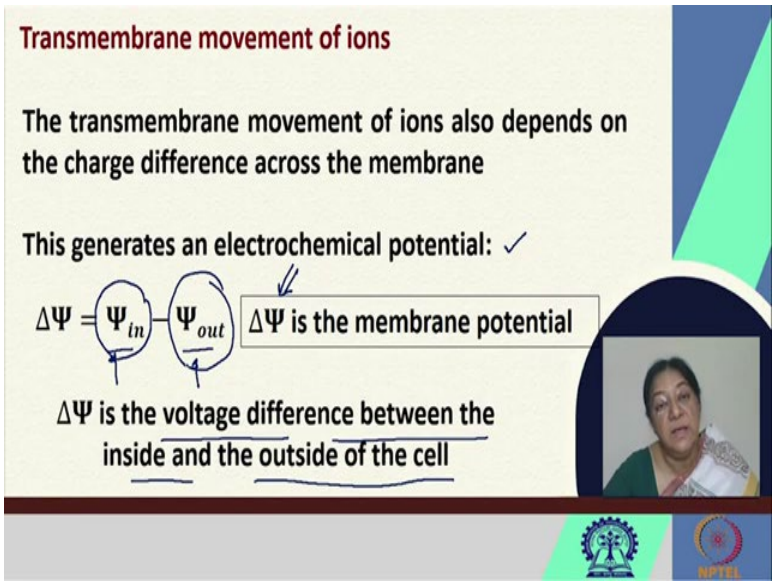

So, the transmembrane movement of ions can therefore be accomplished. It depends upon the charge difference across the membrane. This generates what is called an electrochemical potential, which we will revisit in the next lecture that also will deal with membrane transport.

What we have here is, we have an electrochemical potential inside the cell and outside the cell, the voltage difference between the inside and the outside of the cell is given by this $\Psi_{in} - \Psi_{out} = \Delta\Psi$ .This is our membrane potential and this will change depending upon the ions that are present inside, the ions that are present outside and their transfer.

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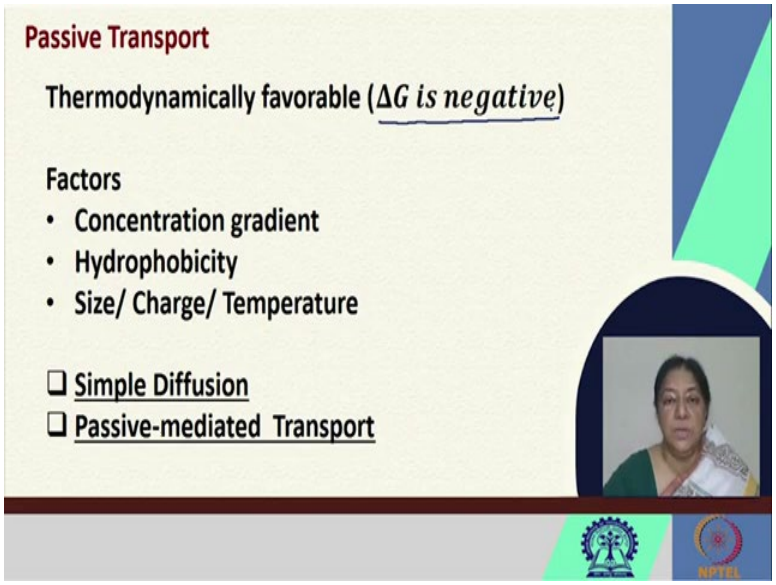

If we look at the transfer in terms of what we call passive transport, this is thermodynamically favorable. We will be looking at the thermodynamics of membrane transport in the next lecture,

where we will see the presence or see how we can accomplish the value or how we can determine the value of ΔG, given the concentration of ions inside and outside the cell.

The factors that are important in this case are the concentration gradients. So we would want to know the concentration of ions, inside the cell and the concentration of the same ions outside the cell to determine the ΔG associated with this.

The hydrophobicity of the specific membrane, the size, charge and temperature are other properties that are important. There could be simple diffusion across the membrane or there could be passive mediated transport, where there will be proteins that would assist the transport of the material from one side of the cell to the other through the plasma membrane.

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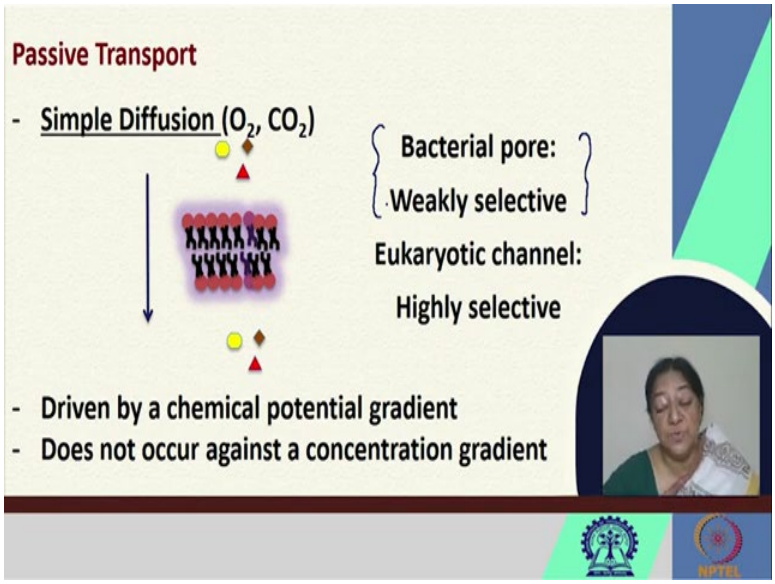

In the case of simple diffusion, where we are looking at just oxygen and carbon dioxide transfer from the transport through the plasma membrane, this is driven by a chemical potential gradient and it does not occur against any concentration gradient. So if we have our membrane and we are looking at the transport of the small molecules, oxygen and carbon dioxide in a simple diffusion method, we would just have this pass through the membrane.

We can have selectivity in some cases bacterial pores can be weakly selective, on the other hand we can have eukaryotic channels, that can be highly selective as we will see later on; specific to ions.

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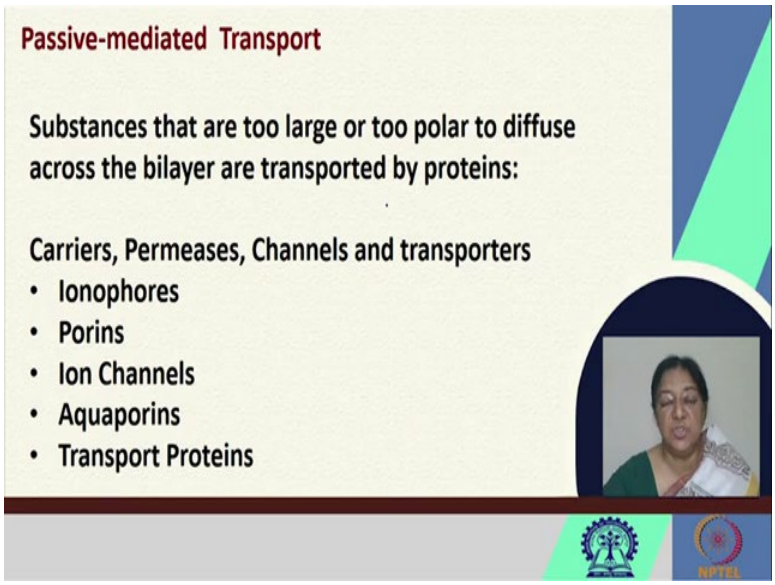

In passive mediated transport, substances that are too large or too polar to just diffuse across the bilayer, are transported by proteins. These proteins are carriers, permeases, channels and transporters and they assist in the transfer of the material and specific molecules across the membrane.

The carriers can be ionophores, they can be porins, they can be specific ion channels, they can be aquaporins and as the name implies, they transport water through the membrane and specific transport proteins.

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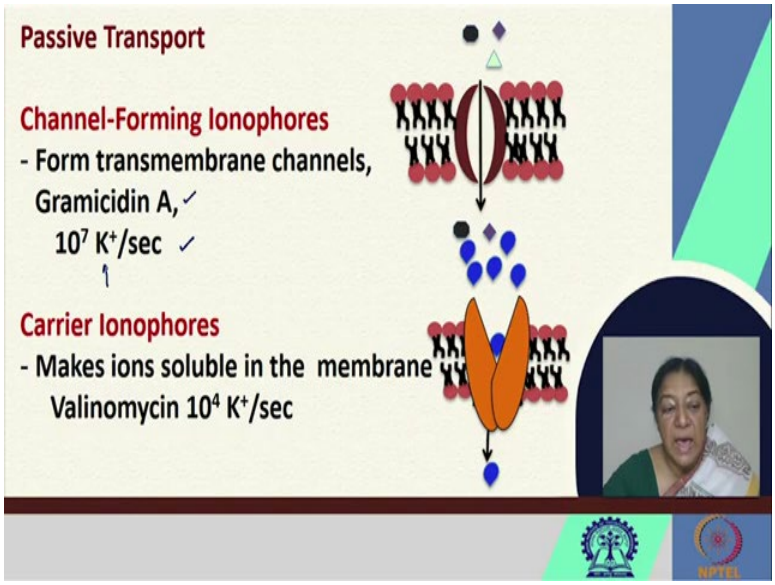

The passive transfer can occur through channel forming ionophores. In this case, there are transmembrane channels formed and if we look at a specific example of gramicidin A, this transfers around 10^7 K^+ ions/sec and this [refer to slide] is the way it does the transfer, where

there is a channel forming ionophore. When we look at a carrier ionophore, it makes ions soluble in the membrane. For example, in valinomycin it allows the transfer in this fashion and in case of valinomycin, 10^4 K^+ ions/sec is the transfer rate.

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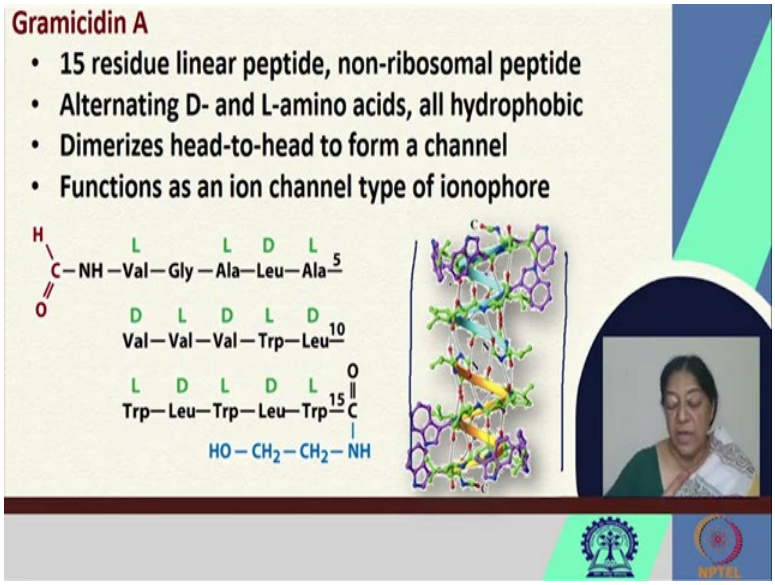

If we look at the structures of these specific types of proteins gramicidin A and valinomycin, we will see that they are quite small. Gramicidin is a 15 residue linear peptide. It is a non-ribosomal peptide, composed of alternating D and L amino acids that are all hydrophobic in nature.

As we saw in the last lecture, we would have likely all hydrophobic amino acids on the surface of the helix. It dimerizes head to head to form a channel through the membrane and it functions as an ion channel type of an ionophore.

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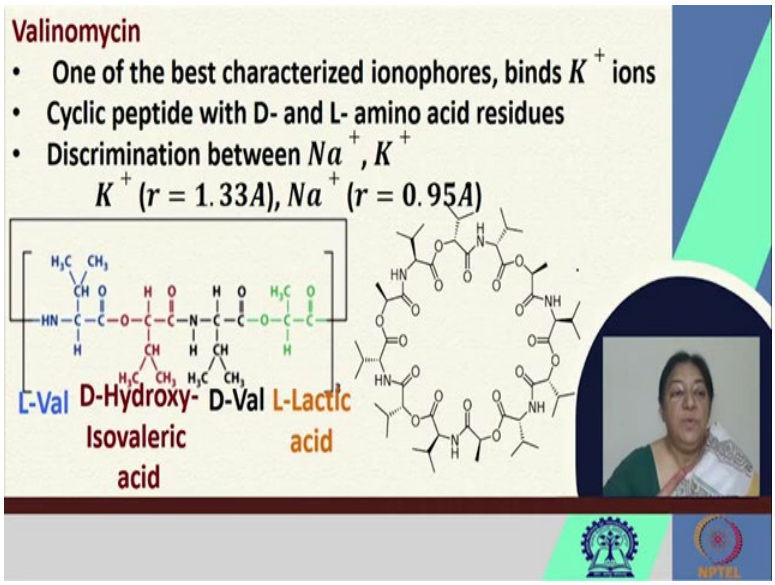

If we look at valinomycin, this is one of the best characterized ionophores. It binds the K^+ ions. It is a cyclic peptide that creates this channel that transports the specific ions and the cavity here [refer to slide] is very specific and it can discriminate between sodium and potassium.

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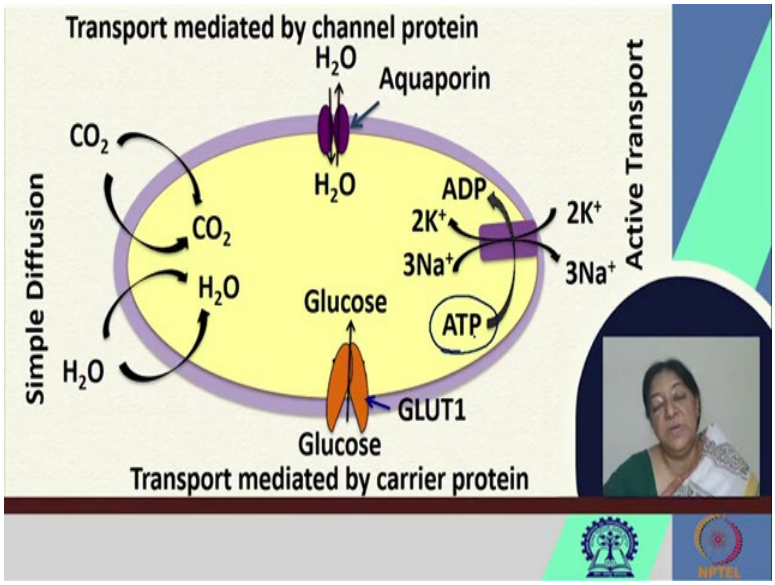

So if we look at the types of transportations possible, we have our cell membrane, we have a cytoplasmic region and we have our external region. We can have simple diffusion, we can have an aquaporin that will transfer the water, we can have a glucose transport mediated by the carrier protein, which we will see in a moment and we could have active transport which we will look at in the next lecture.

This looks at all the different possibilities that are there for the transport through the membrane. If we now look at the specific active transport type, we will see that we will have ATP come into

the scene where we have an ATP content and this energy requirement is there for active transport.

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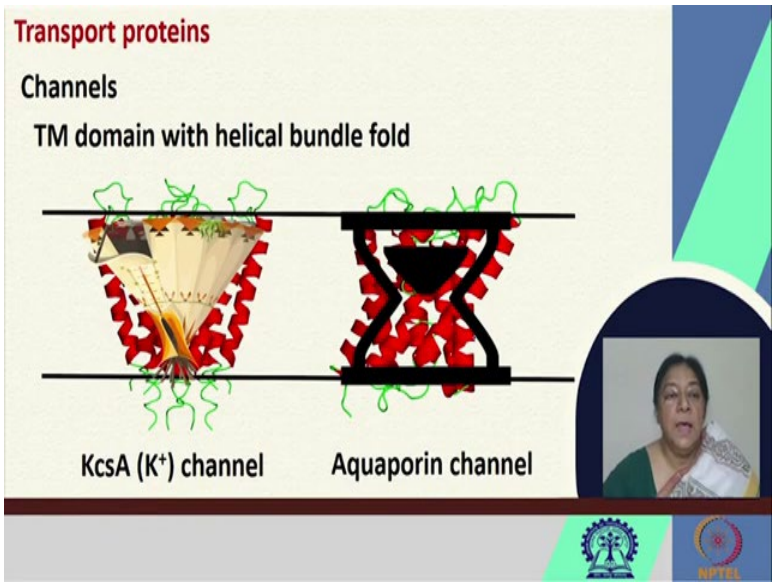

There are specific type of channels that have a transmembrane domain which could be specific for an ion such as the K^+ channel or we could have an aquaporin channel, that would be specific for the transport of water.

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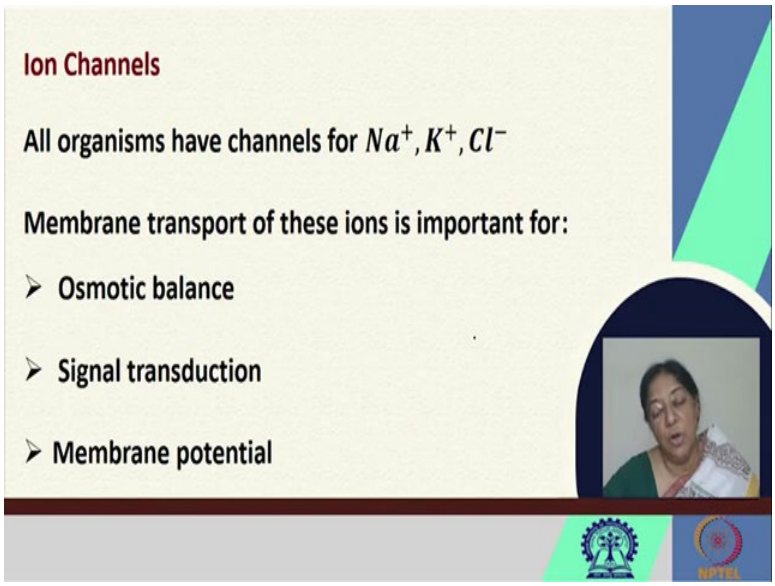

If we look at these ion channels, all of these organisms have channels for sodium, potassium, chloride ions and the membrane transport of these ions is very important to maintain the osmotic

balance for signal transduction and for the specific membrane potential to maintain the resting membrane potential of the membrane.

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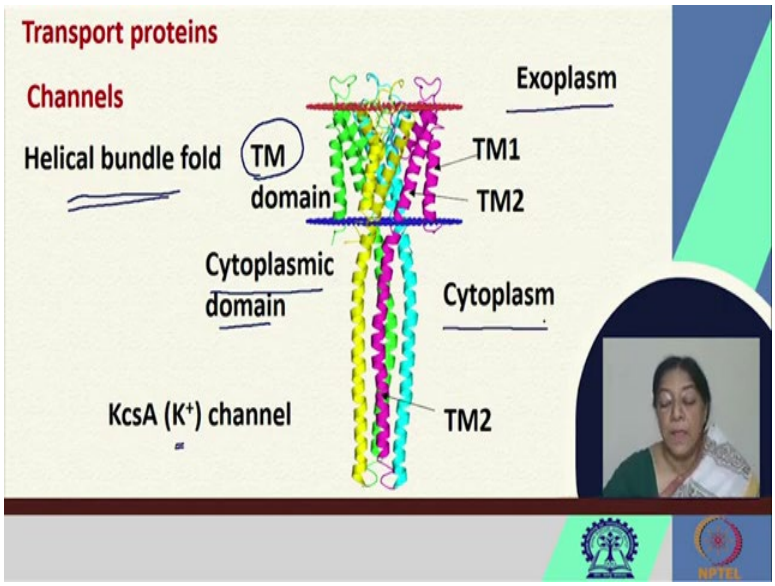

The channels that are these [refer to slide] transport proteins; for example, we have a helical bundle fold as this is called, this being the transmembrane domain. We also have a cytoplasmic domain in this specific example of the K^+ channel, where we have the exoplasm and the cytoplasm here.

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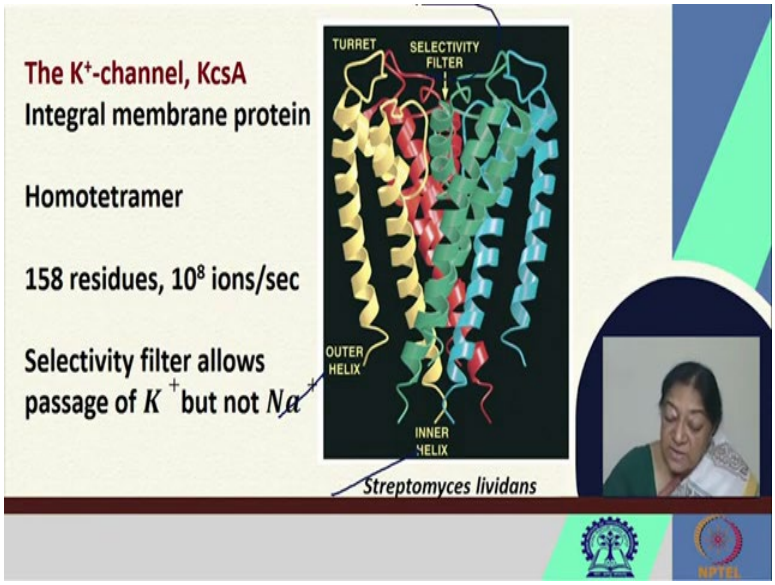

When we look at the K^+ channel, a very interesting protein., we have its structural aspects with the specific helices, where we have an outer helix and we have an inner helix. In this case, this is

an integral membrane protein, a multi-helical protein. It is a homotetramer. It has 158 residues and has the capacity to transfer 10^8 ions/sec and the selectivity filter that is here in the center of the channel, allows the passage of K^+ only and not Na^+.

It works in a very interesting fashion, where the inner pore has small polar OH residues, the residues that contain which that is serine, threonine among them that would hold on to the dehydrated K^+ as it goes through, but not Na^+ because that is not energetically favorable for it. So, when it brings about this K^+ ion, we will see a larger concentration of K^+ inside the cell than the outside of the cell.

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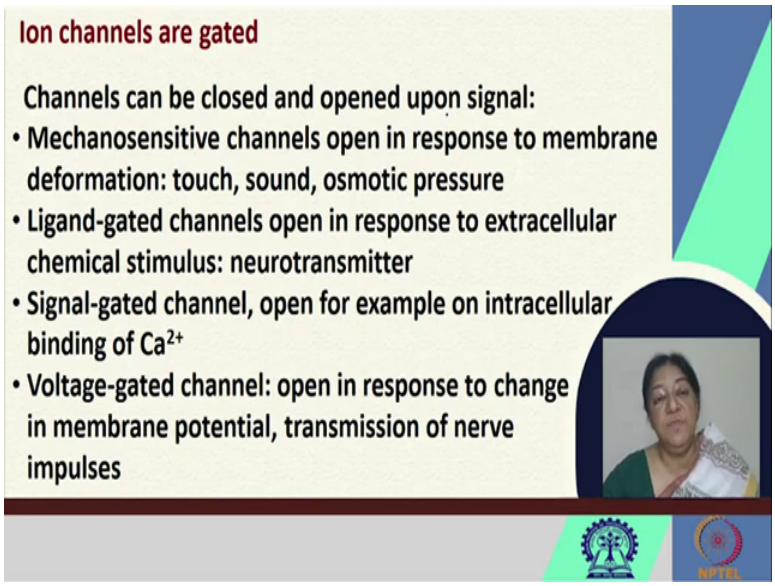

Now, these ion channels are gated. In the sense, that they are closed and open upon specific signals. As we saw these signals can be mechanosensitive in nature that responds to membrane deformation, touch, sound or osmotic pressure.

They could be ligand-gated, they open in response to say a neurotransmitter or they could be signal-gated, they open on the intracellular binding of say calcium and voltage-gated, that could open in response to a change in the membrane potential, which is involved in transmission of nerve impulses.

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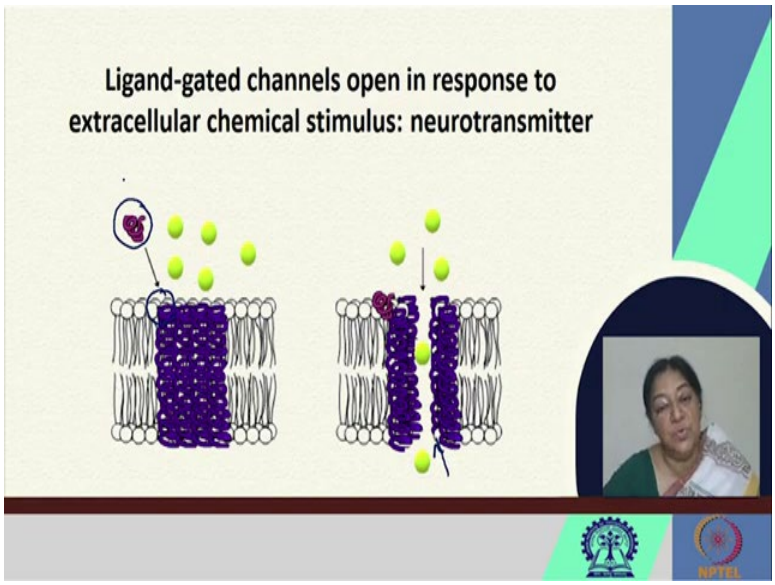

If we look at an example of a ligand-gated channel, opening in response to an extracellular chemical stimulus, we can look at the example of a neurotransmitter binding. So this [refer to slide] is our neurotransmitter, a small ligand molecule that would bind to the specific site associated on the transmembrane protein for it.

Once this binds, then these molecules are not able to transfer through to the membrane; but on binding, this opens up the gate and allows the transfer through the gate. So this transfer is a ligand-gated channel, that opens up only when the ligand is bound to the transmembrane helical protein.

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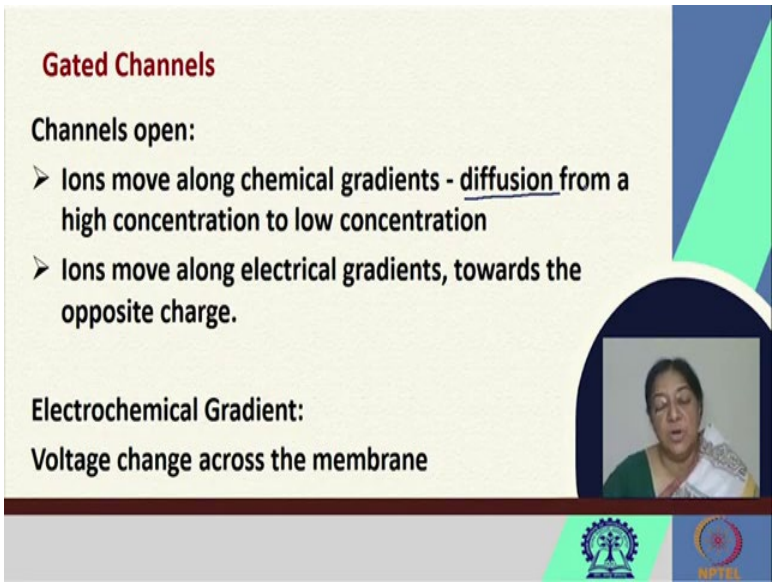

Now for the gated channels, we have the channels open. The ions therefore can move along chemical gradients and the diffusion process will occur from a high concentration to a low

concentration. The ions can also move along electrical gradients and as would be expected, they would move towards the opposite charge. Then we can have an electrochemical gradient, due to the voltage change across the membrane.

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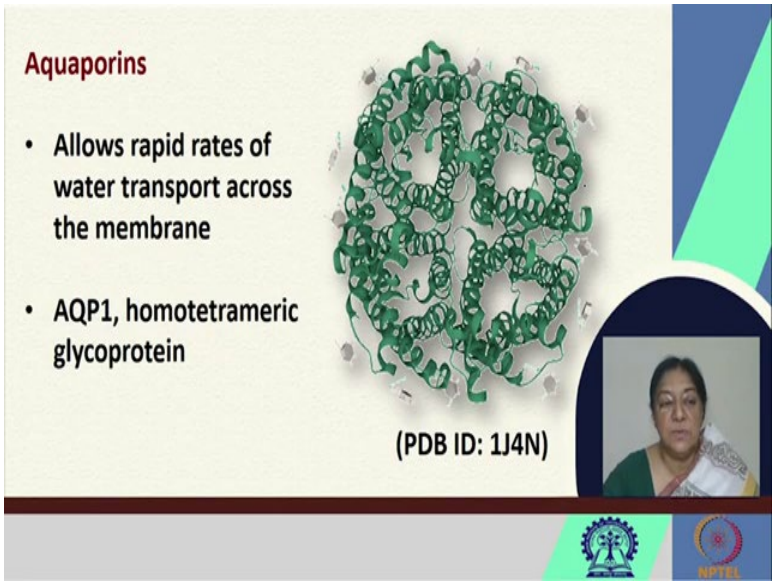

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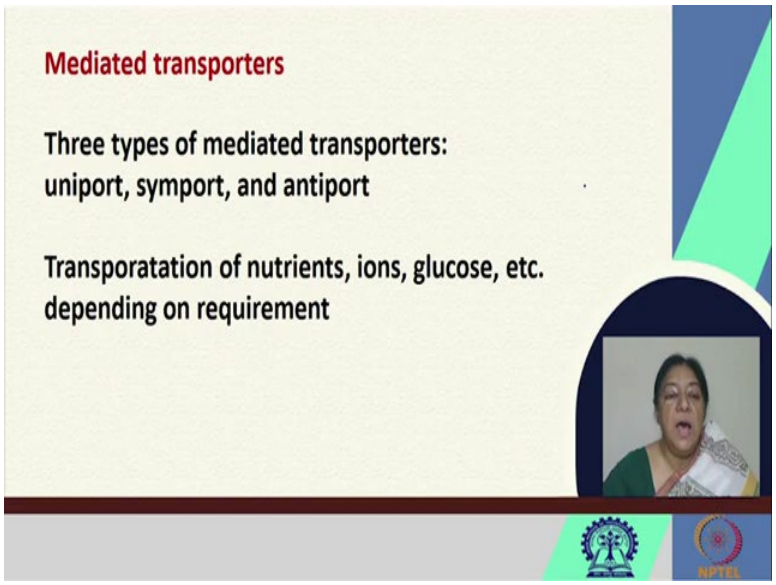

Aquaporins are one such examples that allow the rapid rates of water transport through the membrane. Another type of example are the mediated transporters, where we have mediated transporters, there are three types of mediated transporters. There are uniport types, symport type and antiport type and this again is involved in the transportation of nutrients and ions.

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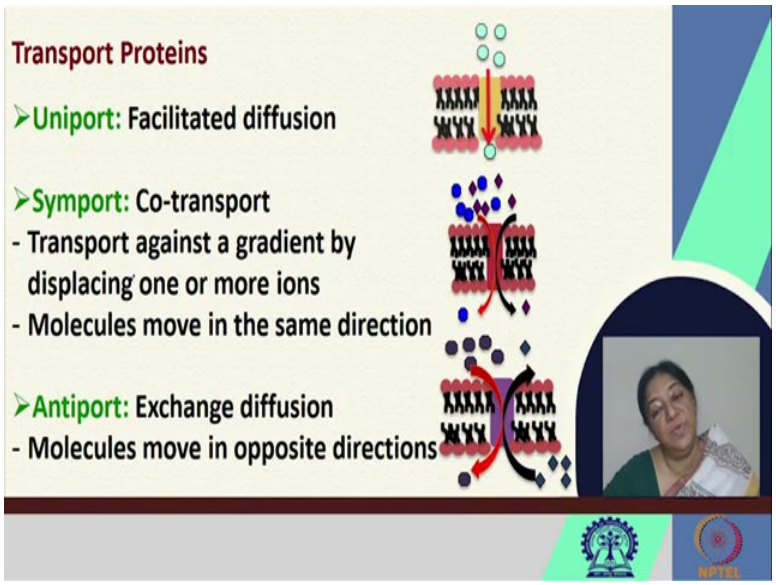

So when we look at transport proteins, we have a uniport that is facilitated diffusion, where we have the membrane and we have facilitated diffusion through the membrane.

We can have a symport type that is has co-transport, where there is the transport against a gradient by displacing one or more of the ions and the molecules move in the same direction. In the antiport type, there is an exchange diffusion and the molecules move in opposite directions. So, we have one moving inside and the other moving outside.

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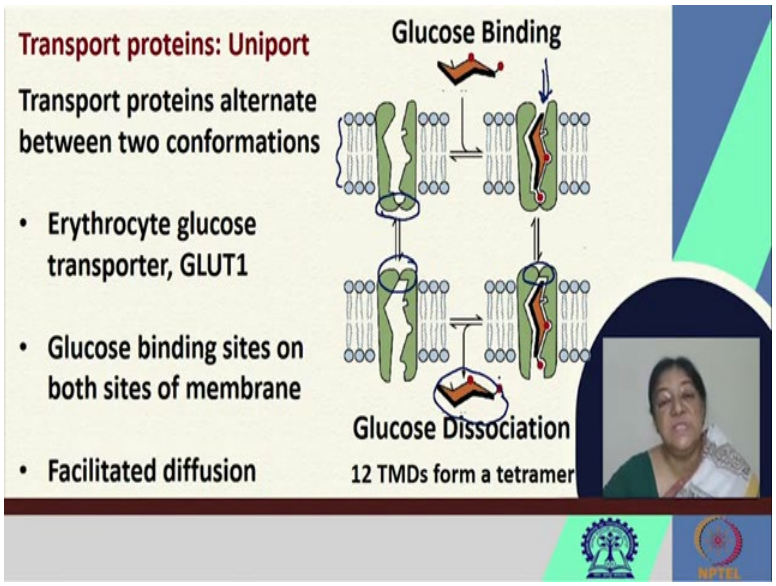

In a uniport example of transport proteins, we will look at the glucose binding protein; the GLUT1 protein, that is an erythrocyte glucose transporter. This [refer to slide] is the lipid bilayer and we have this specific protein embedded here. When glucose binds, there is a specific glucose

binding site and there is a transformation from the outside to the inside, where there would be the possibility of the transfer from the outside to the inside.

In this case, we see this portion closed. It can also intermediate, where we have the top portion closed. Glucose can enter through the top, where we would have glucose binding in this fashion or once it enters, then this can close. This region would open, glucose would be discharged to the other side of the membrane and it would be transported through. So, we have these glucose binding sites on both sides of the membrane and this is facilitated diffusion.

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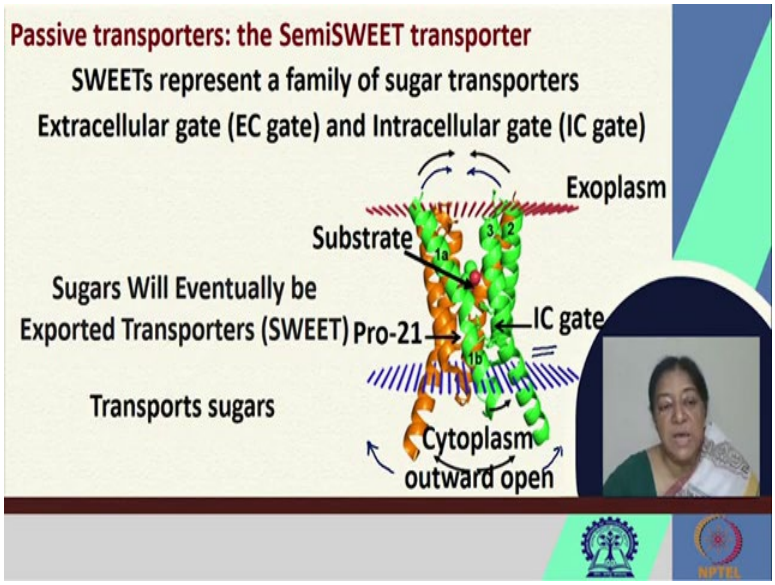

Another such example is called the SWEETs family, that is sugars will eventually be exported transporters. There is a specific extracellular and intracellular gate that opens and closes. So, we can have the closing of the gate at one point and we have the outward opening gate at the other point and on substrate binding, this is facilitated in specific type of sugar transporters. As the name implies these transport sugars.

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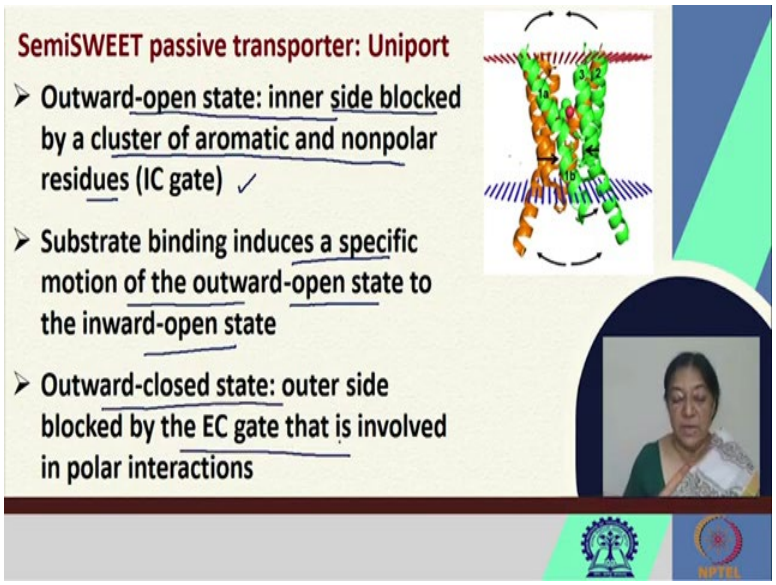

The process is that, there is an outward open state where the inner side is blocked by a cluster of aromatic and nonpolar residues by the intracellular gate. The substrate binding induces a specific motion of the outward open state to the inward open state. So from the outward open state, it moves to an inward open state and then the outward closed state, where this is blocked by the extracellular gate. Whatever transportation is possible, the sugar is bound, the substrate is bound and then it is transferred across the membrane.

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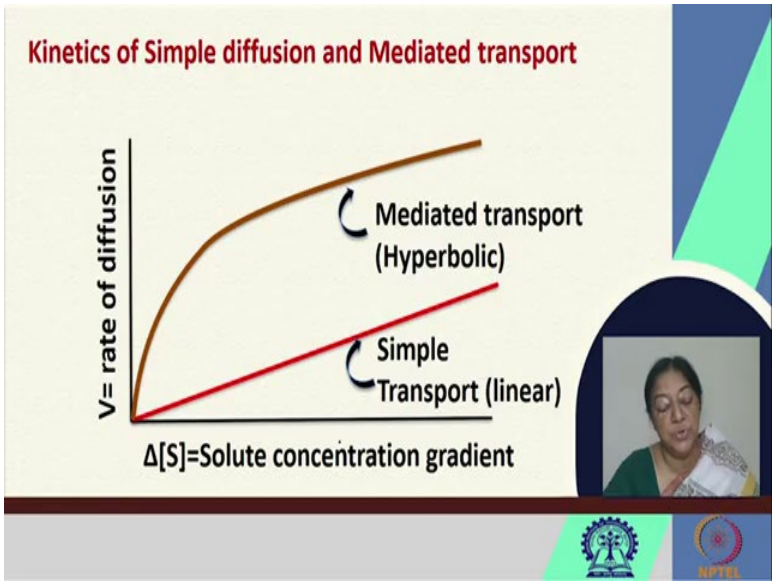

If we look [refer to slide] at the kinetics of the simple diffusion and mediated transport and look at the rate of diffusion, a simple diffusion or a mediated transport would follow a different type of pattern. A mediated transport would follow hyperbolic transport; a hyperbolic fashion type, where we are facilitating this and this would be a simple transport in a linear fashion.

So the assistance due to the mediator, that is the protein here, allows for a better rate of diffusion across the membrane that is facilitated by the protein.

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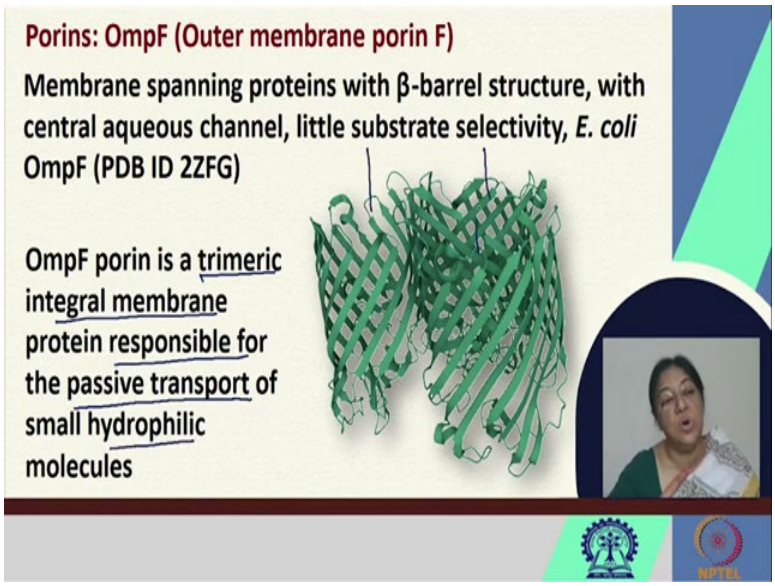

If we look at the porins, the outer membrane porin F, this is also a membrane spanning protein with a β barrel structure, with a central aqueous channel and it has little substrate selectivity in that sense. So, this is a trimeric integral membrane protein that is again responsible for the passive transport of small hydrophilic molecules, through the specific pores that are available due to the β barrel structure.

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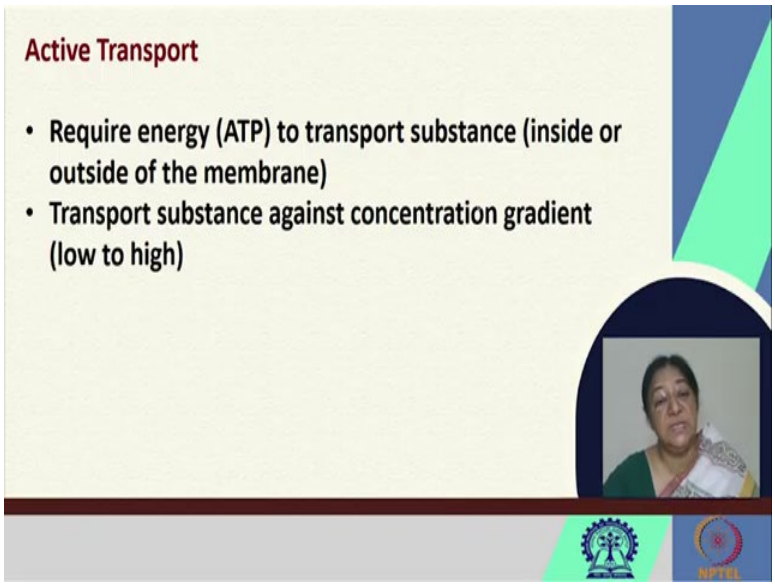

In active transport, which we will visit in the next lecture, it requires energy; ATP to transport the substance, whatever small polar molecules or whatever ions from the inside or outside of the membrane and this transports substances against a concentration gradient.

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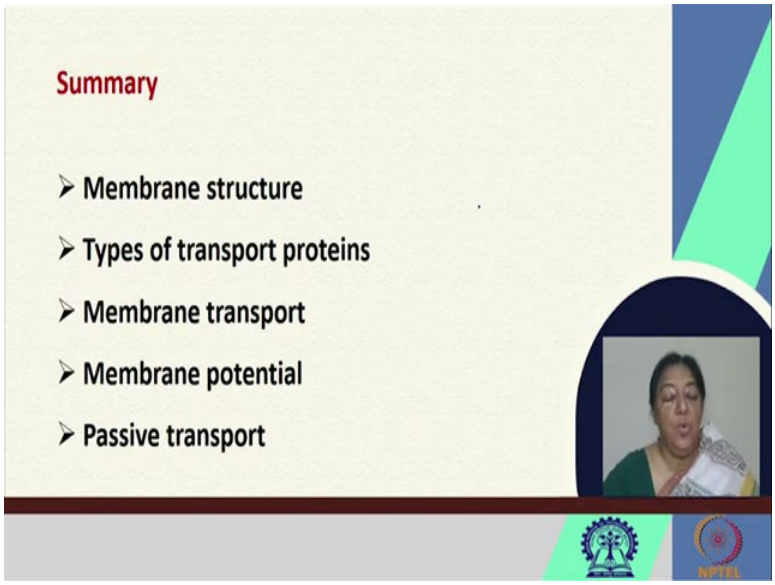

So, what we looked at in this lecture was the general application of the membrane structure and how we can have the different types of transport proteins. These transport proteins are going to assist in the transport of nutrients, small polar and large polar molecules across the cellular membrane. We can have the passive transport or the active transport. We looked at passive transport, where we can have normal diffusion, simple diffusion or we can have mediated diffusion, depending on the type of membrane protein present that would facilitate the transport from one side to the other of the plasma membrane.

We also understood that there is a membrane potential involved and we will look at the membrane potential in a bit more detail to understand the thermodynamics of the membrane transport, thermodynamics of the transfer of material from one side of the membrane to the other.

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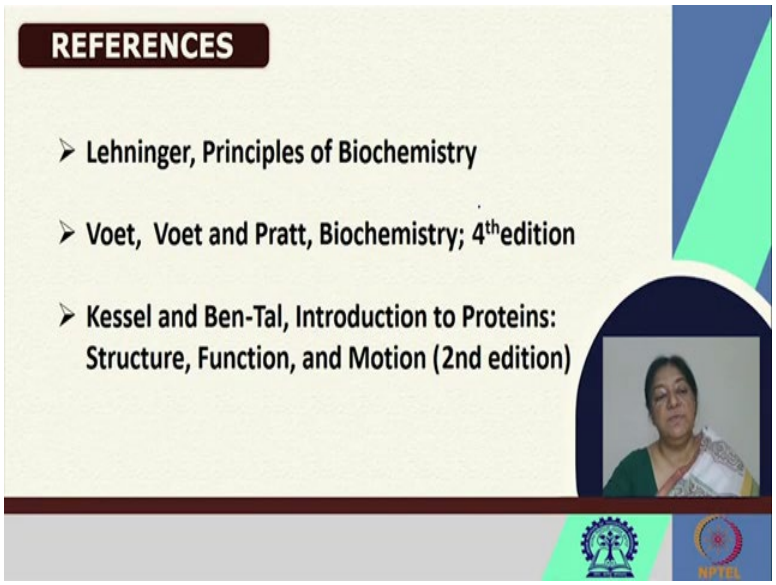

These [refer to slide] are the references.

Thank you.